Research Focus

Found in the crystal: phospholipid ligands for nuclear orphan receptors

Tamas Balla

Endocrinology and Reproduction Research Branch, NICHD, National Institutes of Health, Bethesda, MD 20892-4510, USA

Phospholipids are important components of cellular membranes, contributing to their structural integrity and regulatory functions. Because of these functional properties, phospholipids are often the subject of cell biology and signal transduction studies. Proteins that bind and transport phospholipids between membranes have been described and investigated but few scientists would have entertained the thought of phospholipids acting as ligands for transcription factors. However, the surprising results of recent crystallization studies revealed phospholipid ligands in the binding pockets of members of the nuclear orphan receptor family 5. Their ability to alter transcriptional activity by acting as *bona fide* ligands has been inspirational not only for the transcription factor community, but also for phospholipid researchers.

Introduction

Nuclear receptors (NRs) are one of the largest families of transcription factors and comprise seven subfamilies (NR0–6). NRs interact with specific sequences of DNA found in the promoter regions of target genes. They undergo a ligand-induced conformational change that results in the dissociation of co-repressors and recruitment of co-activators and regulate transcription by allowing the basal transcriptional machinery to effectively interact with the target DNA. NRs have a conserved modular structure consisting of: an N-terminal A/B domain that is a ligand-independent activator (AF-1); a DNA-binding domain (DBD or C domain) followed by a flexible hinge region (D domain); and a ligand-binding domain (LBD or E domain) with a short C-terminal ligand-dependent activator domain (AF-2 or F domain) (Figure 1a). Several proteins have been classified as NRs based on this architecture but, because they have not yet been associated with a ligand, they are called nuclear orphan receptors (NORs). NORs have been found in all seven classes of NRs, and for some a ligand has already been identified [1].

Members of the NR5 family are orphan receptors that belong to one of the four subclasses of the Ftz-F1 subfamily (named for their homology with *Drosophila fushi tarazu* factor-1, the first cloned member of this group). Steroidogenic factor-1 (SF-1, *NR5A1*) and its closest homolog, liver receptor-homolog-1 (LRH-1, *NR5A2*), are the only human proteins that belong to this class. Members of the other subclasses (NR5A3 and NR5A4) are all fish or invertebrate proteins. SF-1 is specifically expressed in steroidogenic tissues and the hypothalamo–hypophyseo–adrenal axis, where it is a master regulator of the development and differentiation of the tissues and also controls steroidogensis and sex determination [2]. By contrast, LRH-1 is expressed in, and regulates the development of, endodermal tissues, such as the liver, exocrine pancreas and intestine, but is also found in the ovary. It has an important role in cholesterol transport, bile acid balance and steroidogenesis [3]. Despite these well-defined and prominent roles, regulatory ligands for these receptors have remained elusive.

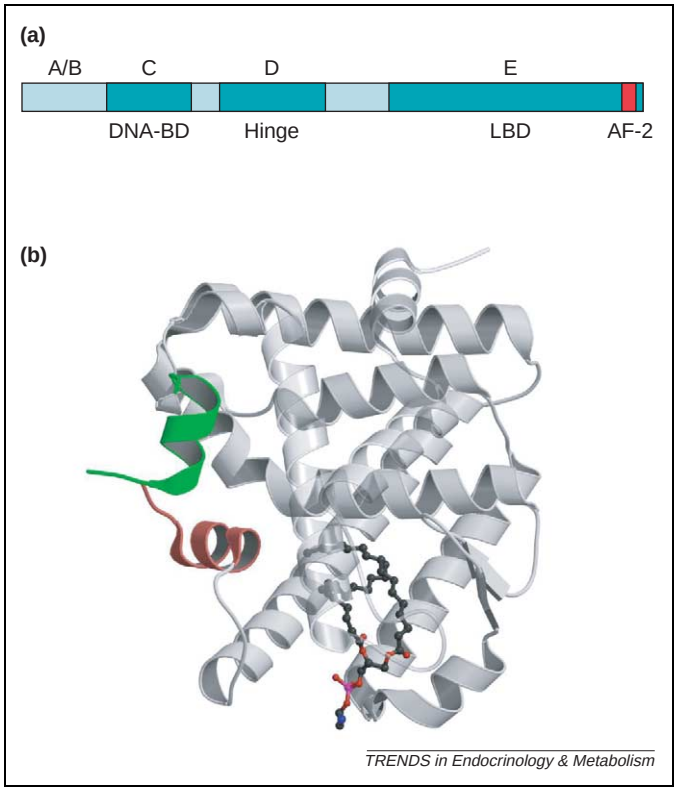

Figure 1. Architecture of nuclear receptors and structure of the SF-1 ligand-binding domain. **(a)** Domain structure of nuclear receptors. The N-terminal A/B domain is a ligand-independent activator (AF-1) and is followed by a DNA-binding domain (DBD or C domain). A flexible hinge region (D domain) connects to the ligand-binding domain (LBD or E domain), which contains the short C-terminal ligand-dependent activator domain (AF-2 or F domain). **(b)** Structure of the SF-1 ligand-binding domain. The phosphatidylethanolamine ligand (shown as a ball-and-stick model) occupies the ligand-binding pocket. The AF-2 segment (red) is in its active conformation, interacting with the first LxxLL motif of the SHP co-activator (green).

Corresponding author: Balla, T. (tambal@box-t.nih.gov).
Available online 28 July 2005

Because of their close involvement in regulating the genes involved in steroid synthesis and transport, NOR candidate ligands have been sought among cholesterol and steroid derivatives. 25-OH cholesterol has been proposed as a ligand for SF-1 [4,5] but oxysterols have failed to activate the receptor in most cell lines [6], making 25-OH cholesterol a controversial candidate regulatory ligand. Other studies suggested that these receptors do not require a ligand to be active. Indeed, based on structural studies, some NORs do not have a ligand-binding pocket, whereas others have a ligand constitutively bound to them. It is believed that the bound ligand only stabilizes the active conformation but does not have a regulatory role [7]. Previous structural studies on the ligand-binding domain of murine LRH-1 indicated that, although it has a deep unoccupied ligand-binding pocket, it also has an active conformation as a monomer without a ligand [8]. Moreover, the addition of bulky side-chains to fill the binding pocket failed to affect the activation potency of murine LRH-1, leading to the conclusion that these NORs are active as monomers and do not require ligand activation [8].

Recent papers by Li *et al.* [9] and Krylova *et al.* [10] have come as a surprise because they report the crystal structures of the ligand-binding domain of human SF-1 and LRH-1 proteins, respectively, and show that their ligand-binding pocket is occupied by phospholipids. Li *et al.* have noted that the ligand- binding pocket of human SF-1 is much larger than that of murine LRH-1 [9] and, although no ligand was added during purification and crystallization, the ligand-binding domain contained an electron density that was identified by mass spectrometry as phosphatidylethanolamine (PtdEtn). The position of the PtdEtn molecule is such that the hydrophobic side-chains occupy the ligand-binding pocket that is lined with hydrophobic molecules, whereas the ethanolamine head-group is partly solvent-exposed (Figure 1b). Importantly, the phospholipid has several direct interactions with the AF-2 helix that stabilize its active conformation. To prove that PtdEtn binding activates SF-1, the authors tested the *in vitro* interaction of recombinant SF-1 with the TIF2 co-activator (LxxLL motif), a faithful indicator of the transcriptional function of the receptor. They found that phospholipids [PtdEtn and phosphatidylcholine (PtdCho)] with C12 and C16 fatty acids could increase the SF-1–TIF2 interaction, whereas longer fatty acids, such as di-C18-PtdCho, act as potent inhibitors. Moreover, selective mutants designed to occupy the ligand-binding pocket strongly interfered with SF-1–TIF2 interactions and reduced the transcriptional activity of the constructs in cell-based assays utilizing SF-1 reporter constructs.

The parallel studies by Krylova *et al.* [10] used three different members of the NR5 orphan receptor family – murine and human SF-1 and human LRH-1 – and came to a similar conclusion. Here, the compound found within the ligand binding pockets was identified as the common bacterial phospholipids PtdEtn and phosphatidylglycerol (PG), PG being the dominant species. Interestingly, human and murine SF-1 and human LHR-1 contained a ligand in a 1:1 stochiometry but only 10% of murine LHR-1 was bound to a ligand, consistent with previous observations by the same group [8]. The structural studies by Krylova *et al.* have identified bacterial phospholipids as the binding partners of NORs, but the authors hypothesized that the ligand in eukaryotic cells might be different. In binding studies utilizing immobilized lipids, they found that phosphorylated phosphatidylinositol (PtdIns) lipids showed prominent binding to the SF-1 and human LRH-1 LBD. Moreover, incubation of the recombinant proteins with specific liposomes revealed that both human and murine SF-1 and, to a lesser degree, human LRH-1, can bind PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2. Although a direct effect of the inositol lipids on the conformation and transcriptional activities of these proteins has not been demonstrated, filling their binding pocket with large residues using site-directed mutagenesis severely compromises their transcriptional activity, suggesting that ligand binding is important for activation.

These intriguing findings raise several important points and questions. The connection between inositol lipids and the genes that these NRs regulate deserves attention and it will be important to determine how phosphoinositides make contact with the NRs. Does the nuclear phosphoinositide system [11] regulate transcription through NR5 proteins or do NR5 proteins acquire their phospholipid ligands outside the nucleus, perhaps from PtdIns transfer proteins [12]? Do NR5 proteins come into direct contact with the membranes to obtain their ligands? These are exciting questions that open a new chapter in phospholipid research, and add transcriptional regulation to the wide range of cellular functions that are regulated by phosphoinositides.

References

1 Zhang, Y. and Dufau, M.L. (2004) Gene silencing by nuclear orphan receptors. *Vitam. Horm.* 68, 1–48

2 Val, P. *et al*. (2003) SF-1 a key player in the development and differentiation of steroidogenic tissues. *Nucl. Recept.* 1, 8

3 Fayard, E. *et al*. (2004) LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis. *Trends Cell Biol.* 14, 250–260

4 Christenson, L.K. *et al*. (1998) Oxysterol regulation of steroidogenic acute regulatory protein gene expression. Structural specificity and transcriptional and posttranscriptional actions. *J. Biol. Chem.* 273, 30729–30735

5 Lala, D.S. *et al*. (1997) Activation of the orphan nuclear receptor steroidogenic factor 1 by oxysterols. *Proc. Natl. Acad. Sci. U. S. A.* 94, 4895–4900

6 Mellon, S.H. and Bair, S.R. (1998) 25-Hydroxycholesterol is not a ligand for the orphan nuclear receptor steroidogenic factor-1 (SF-1). *Endocrinology* 139, 3026–3029

7 Kallen, J.A. *et al*. (2002) X-ray structure of the hRORα LBD at 1.63 A: structural and functional data that cholesterol or a cholesterol derivative is the natural ligand of RORα. *Structure (Camb)* 10, 1697–1707

8 Sablin, E.P. *et al*. (2003) Structural basis for ligand-independent activation of the orphan nuclear receptor LRH-1. *Mol. Cell* 11, 1575–1585

9 Li, Y. *et al*. (2005) Crystallographic identification and functional characterization of phospholipids as ligands for the orphan nuclear receptor steroidogenic factor-1. *Mol. Cell* 17, 491–502

10 Krylova, I.N. *et al*. (2005) Structural analyses reveal phosphatidylinositols as ligands for the NR5 orphan receptors SF-1 and LRH-1. *Cell* 120, 343–355

11 Irvine, R.F. (2003) Nuclear lipid signalling. *Nat. Rev. Mol. Cell Biol.* 4, 349–360

12 Cockcroft, S. (1999) Mammalian phosphatidylinositol transfer proteins: emerging roles in signal transduction and vesicular traffic. *Chem. Phys. Lipids* 98, 23–33

1043-2760/$ - see front matter
doi:10.1016/j.tem.2005.07.006